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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/120,044	07/21/1998	CONCEICAO MINETTI	1758-4036US2	1759

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EXAMINER
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ART UNIT	PAPER NUMBER
1645	37

DATE MAILED: 08/14/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
09/120,044	Minetti et al.
Examiner	**Art Unit**
S. Devi, Ph.D.	1645

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *three* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on *May 8, 2002*.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *35-37, 42-51, 53, and 60-79* is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5) ☒ Claim(s) *43-51* is/are allowed.

6) ☒ Claim(s) *35-37, 42, 53 and 60-79* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☒ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). *36.*
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Request for Continued Examination

1) A request for continued examination under 37 C.F.R 1.114, including the fee set forth in 37 C.F.R 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 C.F.R 1.114, and the fee set forth in 37 C.F.R 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 C.F.R 1.114. Applicants' submission filed on 05/08/02 (paper no. 35) has been entered.

Applicants' Amendment

2) Acknowledgment is made of Applicants' amendment filed 03/13/02 (paper no. 33) which amendment has been entered.

Status of Claims

3) Claims 38-41, 52 and 54-59 have been canceled via the amendment filed 03/13/02.

Claims 35, 53, 60, 62 and 64 have been amended via the amendment filed 03/13/02.

New claims 65-79 have been added via the amendment filed 03/13/2002.

Claims 35-37, 42-51, 53 and 60-79 are under examination.

Prior Citation of Title 35 Sections

4) The text of those sections of Title 35 U.S. Code not included in this action can be found in a prior Office Action.

Prior Citation of References

5) The references cited or used as prior art in support of one or more rejections in the instant Office Action and not included on an attached form PTO-892 or form PTO-1449 have been previously cited and made of record.

Information Disclosure Statement

6) Acknowledgment is made of Applicants' Information Disclosure Statement filed 05/08/02 (paper no. 36). The information referred to therein has not been considered since it is a duplicate of the Information Disclosure Statement filed 02/03/99 (paper no. 8).

Drawings

7) The informal drawings filed in this application are accepted for examination purposes only. Formal drawings will be required when the application is allowed. Applicants are asked to note the changes effected 03 May 2001, particularly the changes to the 'Timing of Corrections':

INFORMATION ON HOW TO EFFECT DRAWING CHANGES

A. Correction of Informalities -- 37 C.F.R 1.85; 1097 O.G. 36

New formal drawings must be filed with the changes incorporated therein. The art unit number, application number (including series code) and number of drawing sheets should be written on the reverse side of the drawings. Applicant may delay filing of the new drawings until receipt of the "Notice of Allowability" (PTOL-37 or PTO-37). If delayed, the new drawings MUST be filed within the THREE MONTH shortened statutory period set for reply in the "Notice of Allowability" to avoid extension of time fees. Extensions of time may be obtained under the provisions of 37 C.F.R 1.136(a) for filing the corrected drawings (but not for payment of the issue fee). The drawings should be filed as a separate paper with a transmittal letter addressed to the Official Draftsperson.

B. Corrections other than Informalities Noted by Draftsperson on form PTO-948.

All changes to the drawings, other than informalities noted by the Draftsperson, MUST be made in the same manner as above except that, normally, a highlighted (preferably red ink) sketch of the changes to be incorporated into the new drawings MUST be approved by the examiner before the application will be allowed. No changes will be permitted to be made, other than correction of informalities, unless the examiner has approved the proposed changes.

Timing of Corrections

Applicant is required to submit acceptable corrected drawings within the three month shortened statutory period set in the "Notice of Allowability" (PTO-37). Within that three month period, two weeks should be allowed for review of the new drawings by the Office. If a correction is determined to be unacceptable by the Office, Applicant must arrange to have an acceptable correction re-submitted within the original three month period to avoid the necessity of obtaining an extension of time with extension fees.

Therefore, Applicant should file corrected drawings as soon as possible.

Failure to take corrective action within the set (or extended) period will result in ABANDONMENT of the application.

Specification - Informalities

8) The specification is objected to for the following reasons:

(a) Effective 23 March 1998, ATCC has a new address: 10801 University Boulevard, Manassas, VA 20110-2209. Amendment to the specification at line 19 on page 44 is suggested to reflect this. It is suggested that Applicants examine the whole specification to make similar correction to the address, wherever it appears.

(b) The use of the trademarks in the instant specification has been noted. For example, see page 66, lines 9 and 26: "Tween 20"; page 52, line 72; page 59, line 20; and page 71, line 20: "Sepharose"; page 52, line 9; page 59, line 28; page 63, line 21: "Superose 12"; page 63, lines 17 and 18; page 64, line 10; and page 64, line 23: "Superdex G-200"; and page 71, line 17: "Superdex 200". The recitation(s) should be capitalized wherever it appears and be accompanied by the generic terminology. Each letter of the trademark must be capitalized. See M.P.E.P 608.01(V) and Appendix l. Although the use of trademarks is permissible in patent applications, the propriety nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks. It is suggested that Applicants examine the whole specification to make similar corrections to the trademarks, wherever such recitations appear.

Objection(s) Moot

9) The objection to claim 39 made in paragraph 17 of the Office Action mailed 09/25/01 (paper no. 30) is moot in light of Applicants' cancellation of the claim.

Objection(s) Withdrawn

10) The objection to claim 42 made in paragraph 17 of the Office Action mailed 09/25/01 (paper no. 30) is withdrawn.

Rejection(s) Moot

11) The rejection of claims 38 and 56 made in paragraph 11(a) of the Office Action mailed

09/25/01 (paper no. 30) under 35 U.S.C. § 112, second paragraph, as being indefinite, is moot in light of Applicants' cancellation of the claims.

12) The rejection of claims 39-41 and 57-59 made in paragraph 11(c) of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 112, second paragraph, as being indefinite, is moot in light of Applicants' cancellation of the claims.

13) The rejection of claims 38 and 56 made in paragraph 12 of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 112, first paragraph, as being non-enabled with regard to the scope, is moot in light of Applicants' cancellation of the claims.

14) The rejection of claims 40, 41, 58 and 59 made in paragraph 13 of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 112, first paragraph, as being non-enabled with regard to the scope, is moot in light of Applicants' cancellation of the claims.

15) The rejection of claims 52, 54 and 55 made in paragraph 15 of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 102(b) as being anticipated over Hill *et al.* (*Infect. Immun.* 62: 757-758, 1994 - Applicants' IDS), is moot in light of Applicants' cancellation of the claims.

16) The rejection of claim 52 made in paragraph 16 of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 103(a) as being unpatentable over Paton *et al.* (*Infect. Immun.* 59: 2297-2304, 1991 - Applicants' IDS) in view of Hill *et al.* (*Infect. Immun.* 62: 757-758, 1994 - Applicants' IDS) and Krishnamurthy *et al.* (*Infect. Immun.* 22: 727-735, 1978, already of record), or Lee *et al.* (*J. Infect. Dis.* 151: 658-664, 1985, already of record), is moot in light of Applicants' cancellation of the claim.

Rejection(s) Withdrawn

17) The rejection of claim 64 made in paragraph 11(b) of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

18) The rejection of claim 35 made in paragraph 14 of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 102(b) as being anticipated by Lock *et al.* (*Microb. Pathogen.* 21: 71-83, 1996 - Applicants' IDS), is withdrawn in light of Applicants' amendment to the base

claim.

19) The rejection of claims 35-37, 53 and 62 made in paragraph 15 of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 102(b) as being anticipated by Hill *et al.* (*Infect. Immun.* 62: 757-758, 1994 - Applicants' IDS), is withdrawn in light of Applicants' amendment to the base claim.

20) The rejection of claims 60-64 made in paragraph 16 of the Office Action mailed 09/25/01 (paper no. 30) under 35 U.S.C. § 103(a) as being unpatentable over Paton *et al.* (*Infect. Immun.* 59: 2297-2304, 1991 - Applicants' IDS) in view of Hill *et al.* (*Infect. Immun.* 62: 757-758, 1994 - Applicants' IDS) and Krishnamurthy *et al.* (*Infect. Immun.* 22: 727-735, 1978, already of record), or Lee *et al.* (*J. Infect. Dis.* 151: 658-664, 1985, already of record), is withdrawn in light of Applicants' amendment to the base claim.

Response to Applicants' Arguments with regard to Scope of Enablement

21) Applicants' arguments have been considered, but are non-persuasive.

Applicants assert that the specification shows that a substitution of proline at position 61, lysine at position 148, or isoleucine at position 195 causes attenuated activity, and that it is reasonable to predict that similar substitutions with hydroxyproline at position 61; arginine or histidine at position 148; and leucine, glycine or alanine at position 195 would also cause an attenuation of haemolytic activity. Applicants cite *In re Angstadt, 537 F.2d 498, 502-03, 190 USPQ 214, 218 (CCPA 1976)* and state that Applicants are not required to disclose every species encompassed by their claims even in an unpredictable art. Applicants further contend that the specification provides guidance as to how to determine whether a specific amino acid substitution would render pneumolysin haemolytically attenuated. However, it should be noted that the attenuated haemolysis is not the only function that is required to be possessed by the claimed polypeptide. Other functions such as partial solubility (currently undefined) as well as retention of an epitope on the modified pneumolysin which epitope binds to an antibody specific to native pneumolysin, and the ability to serve as an effective vaccine and a protein carrier in a conjugate are required to be present in the claimed modified pneumolysin. A relationship is required to exist between the structurally modified polypeptide and the functions recited or

expected. The specification lacks enablement or directions as to how to obtain modified pneumolysins that concomitantly have all these functions while carrying the above-identified amino acid substitution(s). No double, triple, quadruple etc. pneumolysin mutants having up to 20 amino acid substitutions while possessing all the required functions are enabled. The *prima facie* evidence for a clear lack of enablement comes from Applicants' own specification and also from the state of the art as explained below in paragraph 24.

Applicants assert that the breadth of Applicants' claims is not overly-broad since the modified pneumolysin "must comprise at least one specific mutation as described in claim 35". However, the instant specification teaches against a single substitution at several amino acid positions along SEQ ID NO: 3, such as, position 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 255 or 257 by expressly stating that amino acid substitutions at these positions would yield a modified pneumolysin having a haemolytic function that is contrary to what is recited. The simultaneous effect, on partial solubility and immunogenicity/antigenicity, of an amino acid substitution at these positions is unknown. Table 5B most definitively establishes that a substitution at position 243 with any one of the four different amino acids: arginine, valine, glutamic acid or serine, yielded modified pneumolysin species that do not have the required functions, or that would not serve the purpose of the instant invention. Other than modified pneumolysins with a single mutation, pNV103, pNV207, pNV111 and pNV211, the rest of the claimed species could not be used for the purpose desired in the instant invention. In *In re Angstadt, 537 F.2d 498, 502-03, 190 USPQ 214, 218 (CCPA 1976)*, a disclosure of a large number of operable embodiments and the identification of a single inoperative embodiment was found not to render a claim broader than the enabled scope. However, in the instant case, claims encompass a significant number of inoperative embodiments, i.e., modified pneumolysins that possess functions contrary to what is recited, and therefore render the full scope of the claims non-enabled. See paragraph 24 below for a detailed discussion.

Rejection(s) under 35 U.S.C. § 112, First Paragraph

22) Claims 35-37, 42, 53 and 60-79 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the

application was filed, had possession of the claimed invention.

The base claim 35 includes the recitation: the polypeptide is "partially-soluble" (see line 2). Applicants point to page 48, lines 6-8 and state that the limitation "partially-soluble" is in replacement of the element of refoldability "although the instant application states that partial solubility is a probabilistic indicator of refoldability". Applicants conclude that claim 35 does not introduce new matter, and that claim 35 has been amended within the Office's bounds of enablement. The descriptive support for a claim limitation should come from within the bounds of the specification, as originally filed. A review of the specification at page 48, lines 6-8 shows that neither does this part of the specification provides descriptive support for the limitation "partially-soluble", nor does it equate the limitation "partially-soluble" to "refoldability". On the contrary, lines 6-8 on page 48 of the specification state as follows:

> ligated to NdeI- and XhoI-digested pET-17b or pET-24a using T4 DNA ligase. This ligation mixture was then used to transform competent *E. coli* DH5α. Colonies that

Therefore, the above-identified new limitation in the claim(s) is considered to be new matter. *In re Rasmussen*, 650 F2d 1212 (CCPA, 1981). New matter includes not only the addition of wholly unsupported subject matter but also, adding specific percentages or compounds after a broader original disclosure, or even omission of a step from a method. See M.P.E.P 608.04 to 608.04(c).

Applicants are invited to point to the descriptive support in the specification as filed, for the newly added limitation(s), or to remove the new matter from the claim(s).

23) Claims 69-79 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 69, 71, 73, 75 and 78 include the recitation: "comprises a combination of substitutions ...". The recitation, for example, in claim 69 that "the polypeptide comprises a combination of substitutions at residues 17, 18, 61, 66 and 101" encompasses a polypeptide having any combination of substitutions at these positions, such as, 17 plus 18; 17 plus 61; 17 plus 66; 17 plus 101; 18 plus 101; 61 plus 101 and so on. However, there is no descriptive

support within the instant specification for polypeptides having all these various combination(s) of substitutions. Therefore, the above-identified new limitation in the claim(s) is considered to be new matter. *In re Rasmussen*, 650 F2d 1212 (CCPA, 1981). New matter includes not only the addition of wholly unsupported subject matter but also, adding specific percentages or compounds after a broader original disclosure, or even omission of a step from a method. See M.P.E.P 608.04 to 608.04(c).

Applicants are invited to point to the descriptive support in the specification as filed, for the newly added limitation(s), or to remove the new matter from the claim(s).

24) Claims 35-37, 42, 53 and 60-79 are rejected under 35 U.S.C § 112, first paragraph, because the specification, while being enabling for a modified pneumolysin having attenuated hemolytic activity, wherein the pneumolysin polypeptide contains a specific single amino acid substitution at a specific position along the amino acid sequence of SEQ ID NO: 3, such as, valine or isoleucine at position 195; lysine at position 148; and proline at position 61, does not reasonably provide enablement for a partially soluble and haemolytically attenuated modified pneumolysin having a single amino acid substitution at any of the other recited position, or more than one amino acid substitution at more than one of the recited positions, as claimed broadly. The specification does not enable any person skilled in the art to which it pertains, or with which it is most clearly connected, to make and/or use the invention commensurate in scope with these claims.

Instant claims are evaluated based on the *Wands* analysis. Many of the factors regarding undue experimentation have been summarized in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Circ. 1988) as follows:

- The quantity of experimentation necessary (time and expense);
- The amount of direction or guidance presented;
- The presence or absence of working examples of the invention;
- The nature of the invention;
- The state of the art;
- The relative skill of those in the art;
- The predictability or unpredictability of the art; and
- The breadth of the claims

It is noted that on page 5 of the amendment filed 13 March 2002, Applicants state that the

addition to claim 35 of the "element of 'partially-soluble' is in *replacement* of the element of 'refoldability'....." [Emphasis added].

In the instant case, the nature of the invention pertains to modifying the amino acid sequence of a wild type pneumolysin, SEQ ID NO: 3, by substituting one or more of the 20 amino acid residues recited in claim 35. The polypeptide of the amended claim 35 encompasses a modified pneumolysin which carries "at least one amino acid substitution" in at least one of the 20 recited positions along SEQ ID NO: 3 selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255 and 257. The claimed modified pneumolysin thus encompasses one that carries double, triple, quadruple etc., substitutions, or even 10, 15 or 20 amino acid substitutions at the recited positions in SEQ ID NO: 3. The modified pneumolysin encompassed within the scope of the claims is **required** to be partially soluble (i.e., refoldable) and haemolytically attenuated. Furthermore, each of the claimed polypeptide encompassed in the scope of the claim is **required** to retain the function(s) of immunogenicity/antigenicity and the ability to bind with an antibody specific to native pneumolysin, i.e., pneumolysin-specificity, because each modified pneumolysin is meant for use as an individual vaccine and as a protein carrier in conjugate vaccines (see claims 60, 62 and 64, for example). The specification at the end of page 25 states that the modified pneumolysin should possess reduced or no hemolytic activity and be capable of binding an antibody to native pneumolysin. Table 5A shows that modified pneumolysin species, pNV103, pNV207, pNV111 and pNV211, possess one specific amino acid substitution, i.e., Phe195Val, Phe195Ile, Met148Lys and Ser61Pro respectively. The former three have been conjugated to a polysaccharide. These four single mutant pneumolysin species have been shown to be immunogenic and haemolytically attenuated. There is no showing however that pNV103, pNV207, pNV111 and pNV211 mutants are 'partially-soluble'. There is no enablement for other single mutants that are currently encompassed in the scope of the claims which possess the recited functions of partial solubility and attenuated haemolytic activity, and the expected functions of adequate immunogenicity and ability to bind with pneumolysin-specific antibodies. It is not predictable that one or more substitution(s) with any other amino acid at the recited positions, i.e., other than valine or isoleucine substitution at position 195, the lysine substitution

at position 148, and the proline substitution at position 61, would result in a modified pneumolysin that is partially soluble, haemolytically attenuated, sufficiently immunogenic or antigenic, and suitable for use as a vaccine or as a component of a conjugate vaccine. The *prima facie* evidence for the lack of enablement and for unpredictability comes from within the instant specification. For instance, one of the amino acid substitutions recited in claim 35 is made at position 243 of SEQ ID NO: 3. The specification discloses that a single mutation at position 243 of the wild-type pneumolysin, either with arginine, valine, glutamic acid or serine, or a combination of substitutions that includes the position 243, resulted in insoluble (i.e., non-refoldable or not partially soluble) inclusion bodies, and the attempted refolding of the mutant yielded aggregate species (see pages 57 and 58; and Table 5B). Obviously, such an insoluble (i.e., non-refoldable or not partially soluble) pneumolysin would lack the structural, functional and immunogenic and/or biological integrity, and therefore, is not an ideal vaccine candidate or an ideal protein carrier for a conjugate. Furthermore, the specification expressly describes that an amino acid substitution at position 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 255 or 257 of SEQ ID NO: 3 alone does **not** reduce (i.e., attenuate) the hemolytic activity of the pneumolysin, because these sites are **not** associated with decreases in hemolytic activity. See the sentence bridging pages 25 and 26; and lines 1-9 on page 26 of the specification. Yet these amino acid substitution(s) are encompassed in the scope of the claims. The claimed invention is thus not commensurate in scope with the disclosure, because the claims encompass a significant number modified pneumolysins that do not possess the required functions. The art also teaches unpredictability associated with random amino acid substitutions at positions other than those recited in claim 35. For instance, Hill *et al.* (*Infect. Immun.* 62: 757-758, 1994, already of record) demonstrated that an amino acid substitution at any random position over the length of pneumolysin would *not* yield a modified pneumolysin that is haemolytically attenuated, let alone haemolytically attenuated plus partially soluble plus immunogenic or antigenic. For example, Hill *et al.* showed that an amino acid substitution at position 75, 432 or 468 yielded a modified pneumolysin that retained 100% haemolytic activity of the native pneumolysin. An amino acid substitution at position 31 or 127 reduced the haemolytic activity of the pneumolysin only by 25% (see Table 1 of Hill *et al.*). With regard to the toxicity, the same unpredictability has been

established in the art with other bacterial toxins or polypeptides. For instance, the reference of Pizza *et al.* (*Mol. Microbiol.* 14: 51-60, 1994) reflects the unpredictability in the art, as to which of the site directed substitutions at specific positions would eliminate toxicity while at the same time retain structural and functional integrity of a protein such that it acts as an effective biological product. Pizza *et al.* (1994) have shown that amino acid substitutions resulting in mutant proteins, M59, H72 and N192 remained as toxic (+++) as the wild type toxin (see Table 1). Thus, there is neither an enablement within the instant specification as filed, nor is it considered predictable in the art to obtain a modified pneumolysin that has the recited/expected functions while having an amino acid substitution at a position identified on pages 25 and 26 of the specification and recited in claim 35. The required structure-function relationship is lacking for a large number of species bearing the recited amino acid substitution(s).

Furthermore, in the instant application, other than a valine or isoleucine substitution at position 195, a lysine substitution at position 148, and a proline substitution at position 61, no other amino acid substitutions at these specific positions have been shown to yield a modified pneumolysin that is partially soluble (i.e., refoldable), haemolytically attenuated, sufficiently immunogenic or antigenic, and suitable for use as a vaccine or a component of a conjugate vaccine. Not only the position of an amino acid in a polypeptide that is being substituted very critical in retaining the conformational and immunogenic/antigenic integrity and the desired function of the polypeptide, but also the specific amino acid residue that is used for substitution. The *prima facie* evidence for the unpredictability associated with any amino acid substitution at any position also comes from within the instant specification. For instance, one of the amino acid substitutions recited in claim 35 is made at position 243 of SEQ ID NO: 3. The specification explicitly discloses that a substitution at position 243 of the wild-type pneumolysin, with one of the four different amino acids: arginine, valine, glutamic acid or serine, did not yield a 'partially-soluble' (i.e., refoldable) modified pneumolysin as claimed (see pages 57 and 58, and Table 5B). The art has demonstrated this unpredictability with other bacterial toxins or polypeptides as well. For instance, Pizza *et al.* (*Mol. Microbiol.* 14: 51-60, 1994) showed that while the replacement of Val at position 53 of *E. coli* LT polypeptide with Glu or Asp resulted in a non-toxic mutant LT, the substitution with Tyr at the same position 53 caused the collapse and

prevention of the structural assembly of the subunit A of the toxin. Similarly, while the replacement of Val at position 97 of *E. coli* LT with Lys and of serine at position 114 with Lys or Glu yielded non-toxic mutants, the replacement with Tyr at the same position resulted in the collapse and prevention of the structural assembly of the A subunit (see abstract; and pages 56 and 57). Likewise, while the substitution of Arg at position 192 of LT with Gly resulted in a less toxic, adjuvantic mutant LT (see Clements *et al.*, US 6,019,982), the substitution of arginine at the same position 192 with Asn did not reduce the toxicity of LT (see abstract; page 57 and Table 1 of Pizza *et al.*, 1994). Thus, considerable unpredictability is recognized in the art as to which of the amino acid substitution(s) at any specific position would eliminate the toxic or haemolytic effect and at the same time preserve the structural and functional (i.e., immunogenic or biologic) competence of the protein. Therefore, in the instant case, other than a modified pneumolysin bearing a valine or isoleucine substitution at position 195, a lysine substitution at position 148, and a proline substitution at position 61, no other modified pneumolysins having any other amino acid substitution at these specific positions are enabled which modified pneumolysins are concomitantly partially soluble, haemolytically attenuated, sufficiently immunogenic or antigenic and suitable for use as a vaccine or a component of a conjugate vaccine.

Instant claims also encompass multisubstituted pneumolysins. The recitation: "at least one amino acid substitution" in claim 35, means that the claimed polypeptide can have more than one and as many as 20 amino acid substitutions within the pneumolysin of SEQ ID NO: 3. However, there is no enablement for double, triple or quadruple mutants of pneumolysin, or modified pneumolysins having as many as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid substitutions at the recited positions of SEQ ID NO: 3, which modified pneumolysins possess the recited functions of partial solubility and attenuated haemolytic activity, and the expected functions of adequate immunogenicity and ability to bind with pneumolysin-specific antibodies. From the last paragraph of the specification on page 25, it appears that retention of an epitope which is capable of binding an antibody to the native pneumolysin is critical while producing the modified pneumolysins of the instant invention by amino acid substitution. The modified pneumolysins are meant for use as individual vaccines or in conjugation with polysaccharides. The resultant modified pneumolysin with multiple amino acid substitutions is

required to be partially soluble, haemolytically attenuated, immunogenic or antigenic and suitable for use in a vaccine or as a protein carrier in a conjugate vaccine. However, such a multisubstituted pneumolysin having the recited and/or the expected functions is not enabled. Table 5A depicts pneumolysin mutants carrying four or five amino acid substitutions. For example, pNVJ1 mutant carries Lys17Arg, Lys18Asn, Ser61Pro, Asn66Tyr and Ile101Thr substitutions (claim 69); pNVJ45 mutant carries Asp41Gly, Thr172Ala, Phe195Ile and Lys255Gly substitutions (claim 72); pNVJ20 mutant carries Thr63Ser, Val127Glu, Asn128His and Met148Lys substitutions (claim 74); pNVJ22 mutant carries Ile33Thr, Ile46Thr, Leu83Ser, Ser239Arg and Asp257Gly substitutions (claim 76); and pNVJ56 mutant carries Val65Ala, Asp102Gly, Gln189Arg and Phe195Val (claim 79). Table 4 shows that these modified pneumolysins carrying four or five specific amino acid substitutions have attenuated haemolytic activity. However, there is no showing that these modified pneumolysins are 'partially soluble' **and** possess the capacity to bind with an antibody to native pneumolysin, i.e., retain pneumolysin-specificity or pneumolysin-neutralizing ability. It is highly unlikely that such multisubstituted polypeptide species retain antigenic, immunogenic and/or biologic functions, since the art teaches that even a single amino acid substitution can drastically alter the function(s) of a protein or polypeptide. This is critical because the resultant modified pneumolysins carrying four or five, or as many as 20 amino acid residues are meant for use in the instant invention as vaccines or a protein carriers in conjugate vaccines. Although haemolytically attenuated, a polypeptide with altered or abolished immunogenicity is not a suitable vaccine candidate, alone or in combination/conjugation with a polysaccharide. There is no evidentiary support in the instant specification that a modified pneumolysin comprising SEQ ID NO: 3 having more than one amino acid substitution at the recited positions, would be functional as an immunogen, a vaccine, an antigen capable of binding specifically with antibodies to native pneumolysin, or as a protein carrier for use in a conjugate. This is important because modifications of a native polypeptide at multiple positions can potentially alter its conformational, antigenic/immunogenic and biologic integrity, and the use of such an excessively modified polypeptide as an immunogen, a vaccine, an antigen or a protein carrier would potentially induce antibodies that are not directed to the native unmodified polypeptide. It is not predictable that multiple

substitutions in a polypeptide would indeed result in a modified product that remains antigenic, retains the conformational assembly required for its immunogenic functions, and brings about the biologic effects that are desired for, such as attenuated haemolytic activity and partial solubility. As explained above, the specification provides evidence that even a single amino acid substitution at several of the positions recited in claim 35 fails to yield a modified pneumolysin which is soluble and/or haemolytically attenuated. This is important because the art, for example, reflects unpredictability as to which amino acids in a specific peptide, polypeptide or protein can be replaced, and with which other specific amino acid, without adversely affecting the functional properties of that specific peptide, polypeptide or protein. While it is known in the art that substitution of one or more amino acids is possible in a given protein or polypeptide, the exact amino acids within its amino acid sequence where replacements or variations can be made, with a reasonable expectation of success of retaining the peptide's, polypeptide's or protein's functional, i.e., antigenic, immunogenic and biologic competence, is not predictable. A random replacement affecting the epitopic amino acid positions that are critical to the three-dimensional conformational structure and immunogenic/antigenic property of the peptide, polypeptide or protein, would result in a product that is non-functional (i.e., not partially soluble and not sufficiently attenuated haemolysis-wise), and not optimally immunogenic as a vaccine or protein carrier, or not optimally antigenic as a diagnostic composition, because such positions tolerate no or little modifications. For instance, with regard to the specific antibody binding ability, the art reflects that retention or preservation of an epitope within a polypeptide following one or more amino acid substitutions is an unpredictable event irrespective of whether the substitutions are within or outside the epitope. For instance, McGuinnes *et al.* (*Mol. Microbiol.* 7: 505-514, February 1993) teach that "[a] single amino acid change within an epitope, or an amino acid deletion outside an epitope, were both associated with loss of subtype specificity resulting from a change in the predicted conformation at the apex of the loop structure" (see abstract). Similarly, McGuinnes *et al.* (*Lancet* 337: 514-517, March 1991) teach that a point mutation generating a single amino acid change in a P1.16-specific epitope in the VR2 region of the *porA* gene of a strain of *Neisseria meningitidis* of subtype P1.7,16 results in "striking changes in the structural and immunological properties of the class 1 protein" of this isolate (see abstract; and page 514).

Similarly, Houghten *et al.* (New Approaches to Immunization, *Vaccines86*, Cold Spring Harbor Laboratory, p. 21-25, 1986) teach the criticality of one or more amino acid residues and their positions in peptide antigen-antibody interactions. Houghten *et al.* state (see page 24):

> One could expect point mutations in the protein antigen to cause varying degrees of loss of protection, depending on the relative importance of the binding interaction of the altered residue. A protein having multiple antigenic sites, multiple point mutations, or accumulated point mutations at key residues could **create a new antigen** that is precipitously or progressively **unrecognizable by any of the antibodies** in the polyclonal pool. [Emphasis added].

Thus, the art reflects that substitutions of critical residues at specific positions in an amino acid sequence could result in a peptide which may induce an antibody that may not recognize or bind to the native polypeptide. In the instant case, this issue is of importance because multisubstituted pneumolysin species that are encompassed in the scope of the claims are required to be immunogenic/antigenic and biologically competent for use in a vaccine or a conjugate and are required to bind to an antibody specific to native pneumolysin. Clearly, the instant disclosure lacks a showing that the multisubstituted pneumolysin species exemplified in Table 5A are partially soluble (i.e., refoldable), specifically reactive with an antibody specific to native pneumolysin and optimally immunogenic being capable of serving as effective vaccines and effective protein carriers in conjugate vaccines. Furthermore, modified pneumolysins having a combination of substitutions at positions 17, 18, 61, 66 and 101 (claim 69); at positions 41, 172, 195 and 255 (claim 71); at positions 63, 127, 128 and 148 (claim 73); at positions 33, 46, 83, 239 and 257 (claim 75); and at positions 45, 102, 189 and 195 (claim 78) with any amino acid which modified pneumolysins possess the required functions are not enabled.

With regard to the scope of claims 65-79, the claimed polypeptide encompasses pneumolysin mutants having hydroxyproline at position 61; arginine or histidine at position 148; leucine, glycine or alanine at position 195; and arginine, valine, glutamic acid or serine at position 243. However, Applicants did not have possession of any of these modified pneumolysins having the required functions. Applicants assert that the specification shows that a substitution of proline at position 61, lysine at position 148 or isoleucine at position 195 causes attenuated activity, and that it is reasonable to predict that similar substitutions with hydroxyproline at position 61; arginine or histidine at position 148; and leucine, glycine or

alanine at position 195 would also cause an attenuation of haemolytic activity. There is neither any evidence within the instant specification, nor is it predictable that pneumolysins, if so modified, would be hemolytically attenuated **and** partially soluble (i.e., refoldable as described by Applicants on page 5 of the amendment filed 03/13/02) and sufficiently immunogenic and antigenic. The specification demonstrates that even a single amino acid substitution with four different amino acids yielded modified pneumolysin species that were "found **exclusively** in the insoluble fraction as inclusion bodies" [Emphasis added]. See the sentence bridging pages 57 and 58. The state of the art reflects functional unpredictability even with regard to conservative amino substitution. For instance, Lazar *et al.* (*Mol. Cellular Biol.* 8: 1247-1252, 1988) demonstrated that a substitution of Leu with a conservative amino acid residue, such as, Ile or His in the transforming growth factor (TGF) alpha led to a mutant protein with dramatically altered biological activities. Lazar *et al.* stated that they "did not expect that a mutation of Leu to Ile (which have similar sizes and polarities) would cause such a strong effect". See paragraph bridging left and right columns on page 1251; and third full paragraph on page 1251 of Lazar *et al.* Similarly, it is known in the art that a single amino acid substitution even with a functionally equivalent amino acid can alter a peptide's biologic or antigenic function drastically. For instance, Hansen *et al.* (WO 98/06851), a post-filing reference, disclose that when hydrophilic amino acid, aspartic acid (D), is substituted for another hydrophilic amino acid, asparagine (N), at position 3 of the peptide, LDNKYAGKGY, the reactivity of the peptide with the bactericidal 10F3 monoclonal was abolished. See lines 8-14 of WO Hansen *et al.*

Given the lack of adequate disclosure and/or specific guidance in the specification, the art-demonstrated unpredictability, the unpredictability evident from within the instant specification of obtaining modified pneumolysins or polypeptides that are partially soluble, haemolytically attenuated, sufficiently immunogenic for use in a vaccine or conjugate, and antigenic being capable of binding with antibodies to native pneumolysin, the Applicants' own showing that solubility or refoldability of even a single mutant of pneumolysin is non-predictable, the breadth of the claims and the quantity of experimentation necessary, one of ordinary skill in the art could not reproducibly practice the invention commensurate in scope with the claims, without undue experimentation. Therefore, the claims are viewed as not meeting the

scope of enablement provisions of 35 U.S.C § 112, first paragraph.

Rejection(s) under 35 U.S.C. § 112, Second Paragraph

25) Claims 35-37, 42, 53 and 60-79 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(a) Claim 35 is vague, indefinite and confusing in the recitation: "amino acid substitution selected from the group consisting of residues". It is unclear how an amino acid 'substitution' can be selected from the group consisting of 'residues'.

(b) The term "partial" in claim 35 is a relative term which renders the claim indefinite. The term "partial" is not specifically defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. It is unclear what degree of solubility would constitute partial solubility.

(c) Claim 53 is vague and indefinite in the recitation: "polypeptide according to claim 35 wherein the polypeptide is obtained by randomly mutating". Claim 53 depends from claim 35, which recites a modified pneumolysin having an amino acid sequence of SEQ ID NO: 3 which is modified to possess selective substitutions at specific amino acid residues, as opposed to random amino acid residues.

(d) Claim 62 does not have antecedent basis for the recitation: "The" vaccine.

(e) In line 1 of claim 64, it is unclear where does the antecedence come from for the recitation "the polypeptide is a bacterial polysaccharide". Claim 64 depends from claim 63 which recites two polysaccharides; claim 63 already recites a bacterial polysaccharide.

(f) Claim 65 lacks antecedent basis for the recitation: "at least one amino acid substitution". Claim 65 depends from claim 35, which already includes the recitation "at least one amino acid substitution". For proper antecedence, it is suggested that Applicants replace the recitation with --the at least one amino acid substitution--.

(g) Claim 65 is vague and confusing in the recitation "amino acid substitution is a proline or hydroxyproline at residue 61". In order to distinctly claim the subject matter and also for clarity, it is suggested that Applicants replace the recitation with --amino acid substitution is

a proline or hydroxyproline substitution at position 61--.

(h) Claim 66 lacks antecedent basis for the recitation: "at least one amino acid substitution". Claim 66 depends from claim 35, which already includes the recitation "at least one amino acid substitution". For proper antecedence, it is suggested that Applicants replace the recitation with --the at least one amino acid substitution--.

(i) Claim 66 is vague and confusing in the recitation "amino acid substitution is a lysine, arginine or histidine at residue 148". In order to distinctly claim the subject matter and also for clarity, it is suggested that Applicants replace the recitation with --amino acid substitution is a lysine, arginine or histidine substitution at position 148--.

(j) Analogous criticism as described in paragraph (i) above also applies to claims 67 and 68.

(k) In order to distinctly claim the subject matter and for clarity, in claims 65-69, 71-73, 75, 77 and 78, it is suggested that Applicants replace the recitation "residue" with --position-- and "residues" with --positions--.

(l) In order to distinctly claim the subject matter and for clarity, in claims 70, 72, 74, 76 and 79, it is suggested that Applicants replace the recitation "for residue" with --at position--.

(m) Claims 36, 37, 42, 53 and 60-79, which depend directly or indirectly, from claim 35, are also rejected under 35 U.S.C. § 112, second paragraph, as being indefinite, because of the vagueness or indefiniteness identified above in the base claim.

Objection(s)

26) Claim 73 is objected to for lacking a period at the end of the claim.

Remarks

27) Independent claims 43-51 are allowed. Claims 35-37, 42, 53 and 60-79 stand rejected.

28) Papers related to this application may be submitted to Group 1600, AU 1645 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1. The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242, which is able to receive transmissions 24 hours a day and 7 days a week. The RightFax number for submission of before-final amendments is (703) 872-9306. The

RightFax number for submission of after-final amendments is (703) 872-9307.

29) Any inquiry concerning this communication or earlier communication(s) from the Examiner should be directed to S. Devi, Ph.D., whose telephone number is (703) 308-9347. A message may be left on the Examiner's voice mail service. The Examiner can normally be reached on Monday to Friday from 7.15 a.m to 4.15 p.m. except one day each bi-week which would be disclosed on the Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

August, 2002

SD

S. DEVI, PH.D.
PRIMARY EXAMINER